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REMARKS

At the outset, Applicants would like to thank Examiner Shibuya for the very courteous and helpful discussion held with Applicants' representative on May 26, 2004.

During this discussion, the unique structure of the claimed conjugates and the specific claim language used to recite this structure (e.g., "predetermined positions") were explained in great detail. It was noted that the conjugates of the claimed invention, which may be readily constructed via peptide solid phase synthesis (e.g., specification, paragraph bridging pages 11 and 12), contain hapten molecules and marker groups or solid phase binding groups that are incorporated in the carrier at defined and reproducible positions, such that a defined spatial orientation may be achieved between individual groups on the conjugate (e.g., specification, page 6, lines 2-27).

As explained during the discussion, this ability to define and vary distances is advantageous, for example, in reducing signal quenching and for increasing signal strength of certain marker groups (e.g., page 6, lines 18-27). Moreover, this ability is advantageous in that when the claimed conjugates are used as antigens in an immunological method of detection, it is possible to achieve considerably higher sensitivity and precision at a reduced lower detection limit as compared to monomeric and multimeric antigens (e.g., specification, page 5, line 29 to page 6, line 2).

It was also noted during the discussion that the structure of the claimed conjugates is distinct from structures that result from a random or statistical attachment of moieties, such as when moieties are introduced in the presence of multiple equivalent reaction sites (e.g., in the presence of the multiple carboxylate groups typically found in a chain of amino acids).

Claim Rejections – 35 U.S.C. § 112, First Paragraph (Written Description)

The written description rejection of claims 72-77, 81, 83-88, 100, and 107-115 under 35 U.S.C. § 112, first paragraph is respectfully traversed.

1. "Predetermined Positions"

Applicants respectfully submit that the claim terminology "predetermined positions" recited in each of independent claims 72 and 100 unequivocally satisfies the standard for compliance with written description set forth by the Office and by the courts.

In accordance with MPEP 2163.02, "[a]n objective standard for determining compliance with the written description requirement is, 'does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed.' *In re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989)." In the present instance, Applicants claim conjugates "wherein the hapten molecules and the marker groups or solid phase binding groups are coupled to reactive side groups at predetermined positions on the polymeric carrier" (emphasis added). Applicants respectfully submit that the specification clearly and expressly conveys to those skilled in the art that as of the filing date sought, the Applicants were in complete possession of conjugates in which individual groups (e.g., hapten molecules, marker groups, solid phase binding groups) are incorporated in the carrier at defined and reproducible predetermined positions (e.g., specification page 6, lines 2-27). Moreover, the specification clearly and expressly conveys to those skilled in the art that as of the filing date sought, the Applicants were likewise in complete possession of multiple methods for constructing such conjugates (e.g., variants (a) and (b), specification, page 12, line 10 to page 14, line 24). As further explained below in the section addressing the 35 U.S.C. § 112 second paragraph rejections, the meaning of the claim terminology "predetermined positions" would have been abundantly clear to one of ordinary skill in the art in view of the description in the specification.

The written description rejection raised by the previous Examiner against the phrase "predetermined positions" was based on the assertion that "Applicant's claims are directed to conjugates that define the relationship of the entities therein by functional terminology" (Office Action, page 4). Applicants reiterate and emphasize the express mandates of MPEP 2173.05(g), which states that "[t]here is nothing inherently wrong with defining some part of an invention in functional terms" and that "[f]unctional language does not, in and of itself, render a claim improper. *In re Swinehart*, 439 F.2d

210, 169 USPQ 226 (CCPA 1971)." Moreover, as noted during the interview, the claimed invention is not dependent upon—and, therefore, should not be limited to—one or more specific distances between hapten molecules and marker groups or solid phase binding groups. These distances are determined by specific applications and represent mere species within the genus of the claimed invention. The determination of specific distances lies well within the skill of the ordinary artisan in view of the description and examples provided in the specification, and may vary according to the structure and/or identity of the specific antibody to be determined in the immunoassay, the structure and physical properties (e.g., signal properties) of a particular marker group, etc.

Applicants respectfully submit that the clear and unambiguous description provided in the specification clearly and expressly conveys to those skilled in the art that as of the filing date sought, the Applicants were in complete possession of conjugates in which individual groups (e.g., hapten molecules, marker groups, solid phase binding groups) are incorporated in the carrier at defined and reproducible predetermined positions. Applicants further submit that this claim terminology is in full compliance with the above-cited standards set forth by the Office and by the courts.

2. "Non-Immunologically Reactive"

Applicants respectfully submit that the claim terminology "non-immunologically reactive," which is recited in only one of the currently pending claims (i.e., dependent claim 110) unequivocally satisfies the standard for compliance with written description set forth by the Office and by the courts.

As noted above, the fundamental factual inquiry set forth in MPEP 2163.02 for determining compliance with the written description requirement is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, applicant was in possession of the invention as now claimed. In the present instance, Applicants claim a conjugate in which the carrier is "non-immunologically reactive." The specification clearly and expressly conveys to those skilled in the art that as of the filing date sought, the Applicants were in complete possession of non-immunologically reactive carriers—that is, peptide backbones having "an amino acid sequence which does not interfere with the test procedure in the

intended application of the conjugate as an antigen in an immunological method of detection" (specification, page 16, lines 3-8).

For example, the description of the double antigen bridge test provided in the specification (e.g., page 17, line 22 to page 18, line 22) clearly conveys to those of skill in the art that as of the filing date sought, Applicants were in complete possession of conjugates in which the carrier does not interfere with the test procedure when the claimed conjugates are used as antigens in an immunological method of detection—that is, that Applicants were in complete possession of conjugates containing "non-immunologically reactive" carriers. For example, as described in the specification, an antibody to be determined by the double antigen bridge test is exposed to different antigens. The first antigen carries a marker group and the second antigen is bound or capable of being bound to a solid phase. When exposed to the antigens, the antibody to be determined forms a bridge between the solid phase antigen and the antigen carrying the marker group. As one of ordinary skill in the art would readily understand from reading this description, the immunologically reactive components correspond to the antigens (i.e., haptens), both of which may be conjugates according to the claimed invention. The antigens are specific to the antibody to be determined and, as such, qualify as being immunologically active. Likewise, one of ordinary skill would understand that if the carrier backbones of the claimed conjugates were themselves immunologically reactive, this could result in undesired cross-reactivity with the antibodies contained in the sample, thereby leading to diminished selectivity of the conjugate for the antibody to be determined.

As such, the clear and unambiguous description provided in the specification clearly and expressly conveys to those skilled in the art that as of the filing date sought, the Applicants were in complete possession of conjugates containing "non-immunologically reactive" carriers. Thus, this claim terminology is in full compliance with the above-cited standards set forth by the Office and by the courts.

3. Conclusion

For at least all of the reasons set forth above, the specification as filed fully satisfies the written description requirement under 35 U.S.C. § 112, first paragraph. Accordingly, withdrawal of this ground of rejection is respectfully requested.

Claim Rejections - 35 U.S.C. § 112, Second Paragraph

The rejection of claims 72-77, 81, 83-88, 100, and 107-115 under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention, is respectfully traversed.

1. "Predetermined Positions" and "Distances...Defined Thereby"

Applicants respectfully submit that the claim terminology "predetermined positions on the polymeric carrier, such that distances between the hapten molecules and the marker groups or solid phase binding groups are defined thereby" recited in each of independent claims 72 and 100 unequivocally satisfies the standard for definiteness of claim language set forth by the Office and by the courts.

In accordance with MPEP 2171, the determination as to whether a claim particularly points out and distinctly defines the metes and bounds of subject matter to be protected by a patent grant is evaluated in the context of "whether the scope of the claim is clear to a hypothetical person possessing the ordinary level of skill in the pertinent art." In the present instance, Applicants claim conjugates "wherein the hapten molecules and the marker groups or solid phase binding groups are coupled to reactive side groups at predetermined positions on the polymeric carrier, such that distances between the hapten molecules and the marker groups or solid phase binding groups are defined thereby" (emphasis added). As explained above in the section addressing the 35 U.S.C. § 112 first paragraph rejections, the specification clearly and expressly sets forth what is meant by the claim recitation predetermined positions and by the subsequent clarifying recitation that distances between hapten molecules and marker groups or solid phase binding groups are defined by these predetermined positions (e.g., page 6, lines 2-27). Moreover, Applicants respectfully submit that the meaning

and scope of these recitations would have been abundantly clear to one of ordinary skill in the art in view of this description.

Applicants note that the previous Examiner argued that this claim terminology is "deemed to be relative terminology and is also confusing which renders the claims indefinite." For at least the reasons set forth above, Applicants respectfully disagree and request reconsideration of this holding. Moreover, Applicants draw attention to section 2173.01 of the MPEP which states:

> A fundamental principle contained in 35 U.S.C. 112, second paragraph is that applicants are their own lexicographers. They can define in the claims what they regard as their invention essentially in whatever terms they choose so long as the terms are not used in ways that are contrary to accepted meanings in the art. Applicant may use functional language...or any style of expression or format of claim which makes clear the boundaries of the subject matter for which protection is sought. As noted by the court in *In re Swinehart*, 439 F.2d 210, 160 USPQ 226 (CCPA 1971), a claim may not be rejected solely because of the type of language used to define the subject matter for which patent protection is sought.

Applicants draw further attention to section 2173.04 of the MPEP which states:

> Breadth of a claim is not to be equated with indefiniteness. *In re Miller*, 441 F.2d 689, 169 USPQ 597 (CCPA 1971). If the scope of the subject matter embraced by the claims is clear, and if applicants have not otherwise indicated that they intend the invention to be of a scope different from that defined in the claims, then the claims comply with 35 U.S.C. 112, second paragraph.

In view of the clear and unambiguous description provided in the specification, the claim language Applicants have chosen to set forth the subject matter they regard

as their invention—"predetermined positions on the polymeric carrier, such that distances between the hapten molecules and the marker groups or solid phase binding groups are defined thereby"—is in full compliance with the above-cited standards set forth by the Office and by the courts.

2. "Non-Immunologically Reactive"

Applicants respectfully submit that the claim terminology "non-immunologically reactive" recited in dependent claim 110 likewise unequivocally satisfies the standard for definiteness of claim language set forth by the Office and by the courts.

As noted above, the determination as to whether a claim particularly points out and distinctly defines the metes and bounds of subject matter to be protected by a patent grant is evaluated in the context of "whether the scope of the claim is clear to a hypothetical person possessing the ordinary level of skill in the pertinent art." In the present instance, Applicants claim a conjugate in which the carrier is "non-immunologically reactive." Applicants respectfully submit that the specification clearly and expressly defines what is meant by the recitation of "non-immunologically reactive." For example, as described in the specification, this phrase is defined as referring to peptide backbones having "an amino acid sequence which does not interfere with the test procedure in the intended application of the conjugate as an antigen in an immunological method of detection" (specification, page 16, lines 3-8). As noted above, the specification provides ample description to illustrate to one or ordinary skill in the art what is meant by this claim recitation and, moreover, to illustrate why in certain instances it would be desirable for the carriers of the claimed conjugates to be non-immunologically reactive—that is, to prevent undesired cross-reactivity with antibodies to be determined (e.g., page 17, line 22 to page 18, line 22).

Applicants note that the previous Examiner argued that the claim terminology "non-immunologically reactive" is "a relative term which renders the claims indefinite." However, for at least the reasons set forth above, Applicants respectfully disagree and request reconsideration of this holding. In support thereof, Applicants refer again to sections 2173.01 and 2173.04 of the MPEP quoted above.

In view of the clear and unambiguous description provided in the specification, the claim language they have chosen to set forth the subject matter they regard as their invention—"non-immunologically reactive"—is in full compliance with the above-cited standards set forth by the Office and by the courts.

3. "Pharmacologically Active Substances"

Applicants respectfully submit that the claim terminology "pharmacologically active substances" recited in dependent claim 87 unequivocally satisfies the standard for definiteness of claim language set forth by the Office and by the courts.

As noted above, the determination as to whether a claim particularly points out and distinctly defines the metes and bounds of subject matter to be protected by a patent grant is evaluated in the context of "whether the scope of the claim is clear to a hypothetical person possessing the ordinary level of skill in the pertinent art." In the present instance, Applicants claim a conjugate in which hapten molecules may be selected from a group that includes "pharmacologically active substances." The phrase "pharmacologically active" is a well-established and widely used term of art employed routinely in the field of chemistry and pharmacology. As clearly set forth in the specification, "pharmacologically active" substances employed as haptens in the claimed conjugates are useful for the analytical determination of analytes (e.g., specific antibodies) in biological samples (e.g., page 16, line 27 to page 17, line 20), such as by the competitive assays described in Examples 3 and 4 of the specification (e.g., pages 25-28).

One of ordinary skill in the art recognizes that a characteristic feature of "pharmacologically active" substances is that they interact with specific receptors. It is well understood by those of ordinary skill that a non-negligible level of pharmacological activity presupposes a molecular species having sufficiently complementary structure, electronic environment, etc. to those of the receptor. In support of this assertion, Applicants submit attached Exhibit 1, which is excerpted from the learned treatise *Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition* edited by J. G. Hardman, Lee E. Limbird, Perry B. Molinoff, Raymond W. Ruddon, and Alfred Goodman Gilman (McGraw-Hill, New York, 1996, pages 29-41). This Exhibit clearly

demonstrates the level of knowledge available to those skilled in the art vis-à-vis the concept of pharmacological activity. Moreover, Applicants note that as used in dependent claim 87 and in the specification (e.g., page 8, lines 1-18), the phrase "pharmacologically active" is to be understood in its conventional well-established sense.

In addition to the preceding arguments, Applicants note that an extensive list of representative pharmacologically active substances has been provided in the specification, which would further apprise those of ordinary skill in the art what is intended by the claim terminology (e.g., specification, page 8, lines 1-18). By way of example, representative "pharmacologically active substances" in the sense of the claimed invention include antibiotics, opiates, amphetamines, barbiturates, cytostatic agents (e.g., gentamicin, tobramycin, vancomycin, etc.), paracetamol, salicylates, phenytoin, quinine and quinine derivatives, theophyllin etc., hormones and metabolites such as sterols, bile acids, sexual hormones (e.g., estradiol, estriol, testosterone, progesterone, pregnenolone, and derivatives thereof), corticoids (e.g., cortisol, corticosterone, cortisone, and derivatives thereof), cardenolides and cardenolide-glycosides (e.g., digoxin, digoxigenin, strophanthin, bufadienolides, etc.), steroid-sapogenines, steroid alkaloids, peptide hormones, creatinine, thyroid hormones (e.g., T_3, T_4), neurotransmitters (e.g., serotonin, choline, γ-aminobutyric acid), vitamins and mediators such as prostaglandins, leucotrienes, leucoendiines, and thromboxanes.

Applicants respectfully submit that in view of the clear and unambiguous description and the representative examples provided in the specification, and in further view of the well-established definitions routinely employed in the art, the claim language they have chosen to set forth the subject matter they regard as their invention—"pharmacologically active substances"—is in full compliance with the above-cited standards set forth by the Office and by the courts.

4. Conclusion

For at least all of the reasons set forth above, the present claims are not indefinite. Accordingly, withdrawal of this ground of rejection is respectfully requested.

Claim Rejections – 35 U.S.C. § 102

1. *Tam* (U.S. Patent No. 5,229,490)

The rejection of claims 72, 74-75, 86-88, 100, 107, and 110-111 under 35 U.S.C. § 102(b) as being anticipated by *Tam* is respectfully traversed.

Each of independent claims 72 and 100 recites a conjugate comprising hapten molecules and 1-10 marker groups or solid phase binding groups. In addition, each of independent claims 72 and 100 recites that the reactive side groups by which hapten molecules and marker groups or solid phase binding groups are coupled to a polymeric carrier are amino groups and/or thiol groups. As explained below, no interpretation of the multiple antigen peptide systems described in *Tam* simultaneously satisfies both of the above-identified claim requirements.

If *arguendo* the peptide antigens described in *Tam* were regarded as hapten molecules in the sense of the claimed invention, and the diagnostic moiety described in *Tam* were regarded as marker groups in the sense of the claimed invention, then *Tam* still fails to teach or suggest a carrier that simultaneously contains both a hapten molecule (e.g., a peptide antigen) and a marker group. Rather, as is evident from the description in *Tam* (e.g., col. 10, lines 27-34), the diagnostic agent described in *Tam* is intended to be used as an alternative to the peptide antigen and the carriers described therein would not contain both a peptide antigen and a detectable marker at the same time. For example, *Tam* states that: "This invention has been described principally as it is applied to the production of vaccines based on peptide antigens. However, as will be apparent to those skilled in the art, it is not limited to such products. For example, the core molecule could be used as a carrier for ... a diagnostic agent" (col. 10, lines 27-34).

Tam likewise does not teach or suggest a carrier that simultaneously contains both a hapten molecule and a solid phase binding group, wherein the hapten molecule and the solid phase binding group are coupled to reactive amino and/or thiol side groups on the polymeric carrier (N.B., within the meaning of the claims, Applicants note that reactive amino side groups include both the N-terminus of a peptide as well as the individual amino side groups). In the Office Action (pages 10-11), the previous Examiner stated:

> The conjugates of Tam are made on a solid support via coupling at one end of the molecule (at the –OH moiety of the 1st amino acid of the carrier) and thus have a "solid phase binding group" of –OH.... Thus, the conjugate of Tam in Figure 1 has 8 "haptens" (peptide antigens) and one "solid phase binding group" (-OH).

If *arguendo* the terminal Gly-OH residue described in *Tam* were regarded as a solid phase binding group in the sense of the claimed invention, as suggested by the previous Examiner, then the claim requirement that solid phase binding groups be coupled to the polymeric carrier by amino groups and/or thiol groups would not be satisfied.

Claims 74-75, 86-88, 107, and 110-111 depend from claim 72. As such, the rejection of these claims over *Tam* is overcome for the reasons given immediately above. Moreover, each of these dependent claims contains additional limitations making them further distinguishable from *Tam*.

For at least the reasons set forth above, the claimed invention is neither anticipated by nor would have been obvious in view of *Tam*. Accordingly, withdrawal of this ground of rejection is respectfully requested.

2. *Rose et al.* (US 6,001,364)

The rejection of claims 72, 74-76, 86-88, 100, 107, and 110-111 under 35 U.S.C. § 102(e) as being anticipated by *Rose et al.* is respectfully traversed.

Each of independent claims 72 and 100 recites that the reactive side groups by which hapten molecules are coupled to a polymeric carrier are amino groups and/or thiol groups. As explained below, no interpretation of the hetero-polyoxime compounds described in *Rose et al.* satisfies the above-identified claim requirement.

Rose et al. does not teach or suggest a carrier that contains hapten molecules coupled to reactive amino and/or thiol side groups on the polymeric carrier. However, in the Office Action (page 14), the previous Examiner states:

> Six peptide antigens (i.e. hapten) moieties are attached to the "baseplate" molecule of Rose et al in Figure 1.... The

haptens are attached to reactive amino groups (Lys and amine terminus) of the carrier.

If *arguendo* the 6 peptide COSMs (i.e., complementary orthogonal specifically active molecules) of sequence KLEEQRPERVKG described and shown in Figure 1 of *Rose et al.* were regarded as haptens in the sense of the claimed invention, as suggested by the previous Examiner (page 14, last two lines), then the claim requirement that the haptens be coupled to the polymeric carrier by amino groups and/or thiol groups has not been satisfied.

Careful analysis of the structures described in *Rose et al.* reveals that the ε-amino groups of the lysine residues have been substantially modified in order to provide suitable "oxime-forming complementary orthogonal reactive groups." For example, *Rose et al.* states that "[p]referred common residues to carry oxime forming groups can be those which...can easily be modified by conventional methods...[p]referred are lysine and ornithine which are very easily acylated and can be modified by reductive alkylation (e.g., col. 8, lines 20-26).

The principal oxime-forming complementary orthogonal reactive groups described in *Rose et al.* correspond to amino-oxy-acetyl ("AOA") and glyoxylyl ("GXL") groups. Thus, oxime-forming complementary orthogonal reactive groups suitable for oxime formation are obtained by chemical modification of the amino acid residues of lysine. *Rose et al.* describes one such chemical modification as follows:

> When the baseplate is formed from amino acids, the peptide sequence of a baseplate structure can be synthesized by routine solid phase peptide synthesis ("SPPS") and, while the peptide is still attached to the solid phase, Boc-amino-oxyacetic acid (Boc-AOA) in an activated form such as the N-hydroxysuccinimide ester can be added to the nascent peptide chain. For example, the baseplate structure can consist of a peptide having five reactive groups such as five lysine residues. Boc-AOA N-hydroxysuccinimide ester can react with each of the ε-amino groups of the lysine residues, as well as the N-terminus α-amino group if left unprotected,

to form the baseplate structure which, in this example, would contain an ε-AOA-pentalysine sequence and an AOA group at the N-terminus, if the N-terminus α-amino group was intentionally acylated. (col. 11, lines 8-22)

Rose et al. describes a second such chemical modification as follows:

> Alternatively, Boc-serine(benzyl)-OH in an activated form such as the N-hydroxysuccinimide ester can be used to form the complementary orthogonal chemically reactive group on the baseplate structure. Boc-serine(benzyl) N-hydrosuccinimide ester reacts with the ε-amino groups of the five lysine residues, as well as the N-terminus α-amino group if desired, to form a precursor baseplate containing ε-Ser-pentalysine and an N-terminus α-Ser group. Treatment of the precursor baseplate with a mild oxidizing agent, such as periodate at pH 7, will convert ε-Ser-pentalysine and, if present, the α-Ser-N-terminus to ε-GXL-pentalysine and an α-GXL-N-terminus, respectively, thus producing a hexa-GXL-baseplate structure. (col. 11, lines 23-35)

Once the amino groups of the lysine residues have been appropriately modified, an oximation reaction may then be performed to attach a COSM having a complementary reactive group (e.g., col. 11, lines 49-51).

In short, *Rose et al.* does not teach or suggest at least one element recited in each of independent claims 72 and 100—namely, that hapten molecules are coupled to reactive amino and/or thiol side groups on the polymeric carrier.

Claims 74-76, 86-88, 107, and 110-111 depend from claim 72. As such, the rejection of these claims over *Rose et al.* is overcome for the reasons given immediately above. Moreover, each of these dependent claims contains additional limitations making them further distinguishable from *Rose et al.*

For at least these reasons, the claimed invention is neither anticipated by nor would have been obvious in view of *Rose et al.* Accordingly, withdrawal of this ground of rejection is respectfully requested.

Claim Rejections - 35 U.S.C. § 103

1. *Tam* (U.S. Patent No. 5,229,490)

The rejection of claims 72, 74-75, 81, 86-88, 100, and 107-111 under 35 U.S.C. § 103(a) as being unpatentable over *Tam* is respectfully traversed.

Tam could not have rendered the claimed invention obvious because *Tam* does not teach or suggest the entire combination of elements recited in the claimed invention, as noted above. By way of example, *Tam* does not teach or suggest hapten molecules and marker groups or solid phase binding groups that are coupled to a polymeric carrier by reactive amino groups and/or thiol groups. Thus, Applicants respectfully submit that the claimed invention is neither anticipated by nor would have been obvious in view of *Tam*. Accordingly, withdrawal of this ground of rejection is respectfully requested.

2. *Rose et al.* (US 6,001,364)

The rejection of claims 72-76, 81, 86-88, 100, and 107-111 under 35 U.S.C. § 103(a) as being unpatentable over *Rose et al.* is respectfully traversed.

Rose et al. could not have rendered the claimed invention obvious because *Rose et al.* does not teach or suggest the entire combination of elements recited in the claimed invention, as noted above. By way of example, *Rose et al.* does not teach or suggest hapten molecules that are coupled to a polymeric carrier by reactive amino groups and/or thiol groups. Thus, Applicants respectfully submit that the claimed invention is neither anticipated by nor would have been obvious in view of *Rose et al.* Accordingly, withdrawal of this ground of rejection is respectfully requested.

Claim Rejections - 35 U.S.C. § 112, First Paragraph (New Matter)

The rejection of claims 72-77, 81, 83-88, 100, and 107-115 under 35 U.S.C. § 112, first paragraph as containing new matter is respectfully traversed.

As noted during the interview, the recitation in independent claims 72 and 100 that the carrier comprises "a minimum of 5 and a maximum of 100 monomeric units" is fully supported by the description in the specification (e.g., page 6, lines 29-32), which states that "[t]he polymeric carrier molecule which forms the backbone of the conjugate has a maximum length of 100 monomeric units preferably of 3-80 monomeric units and especially preferably of 5-60 monomeric units.

As further noted during the interview, the Markush-style recitation in the previous version of independent claims 72 and 100 that the reactive side groups are selected from the group consisting of amino groups, thiol groups, "and a combination thereof" was fully supported by the description in the specification (e.g., page 9, lines 11-12), which states that "[t]he hapten molecules and marker or solid phase binding groups are preferably coupled to the carrier chain via reactive amino or/and thiol side groups" (emphasis added). Nevertheless, to comply with the present Examiner's preference as expressed during the interview, the Markush-style recitation has been replaced with the equivalent language "wherein the reactive side groups are amino groups and/or thiol groups." The newly added language has *ipsissima verba* support in the specification.

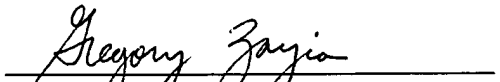
For at least these reasons, Applicants respectfully submit that claimed invention is fully supported by the description in the specification, and that no new matter was introduced with the Response filed December 9, 2003. Accordingly, withdrawal of this ground of rejection is respectfully requested.

Conclusion:

In view of the Amendments and Remarks set forth above, the application with the claims as presently amended is in condition for allowance. Early notification to such effect is earnestly solicited.

If for any reason the Examiner feels that a further interview would be helpful, it is respectfully requested that the Examiner contact the undersigned agent directly at (312)-321-4257.

Respectfully submitted,



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GOODMAN & GILMAN'S The PHARMACOLOGICAL BASIS OF THERAPEUTICS

Ninth Edition

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CHAPTER 2

PHARMACODYNAMICS

Mechanisms of Drug Action and the Relationship Between Drug Concentration and Effect

Elliott M. Ross

This chapter provides an introduction of the concept of receptors, the structural and functional families of receptors, and the interplay between the diverse signaling pathways activated by receptor occupancy. These introductory concepts are amplified in subsequent chapters detailing the structure and function of receptors for individual drug groups. The latter half of the chapter describes the historical development of receptor theory and presents means for quantifying receptor activation by agonists and blockade by antagonists. The functional relevance of partial agonists and inverse antagonists also is described as a prelude to the intentional development of mechanistically diverse drugs via classical or new combinatorial strategies.

Pharmacodynamics can be defined as the study of the biochemical and physiological effects of drugs and their mechanisms of action. The objectives of the analysis of drug action are to delineate the chemical or physical interactions between drug and target cell and to characterize the full sequence and scope of actions of each drug. Such a complete analysis provides the basis for both the rational therapeutic use of a drug and the design of new and superior therapeutic agents. Basic research in pharmacodynamics also provides fundamental insights into biochemical and physiological regulation.

MECHANISMS OF DRUG ACTION

The effects of most drugs result from their interaction with macromolecular components of the organism. These interactions alter the function of the pertinent component and thereby initiate the biochemical and physiological changes that are characteristic of the response to the drug. This concept—now obvious—had its origins in the experimental work of Ehrlich and Langley during the late nineteenth and early twentieth centuries. Ehrlich was struck by the high degree of chemical specificity for the antiparasitic and toxic effects of a variety of synthetic organic chemicals. Langley noted the ability of the South American arrow poison, curare, to inhibit the contraction of skeletal muscles caused by nicotine; however, the tissue remained responsive to direct electrical stimulation. The term *receptor* was coined to denote the component of the organism with which the chemical agent was presumed to interact.

The statement that the receptor for a drug can be any functional macromolecular component of the organism has several fundamental corollaries. One is that a drug potentially is capable of altering the rate at which any bodily function proceeds. Another is that drugs do not create effects, but instead modulate functions.

Drug Receptors

At least from a numerical standpoint, proteins form the most important class of drug receptors. Examples are the receptors for hormones, growth factors, and neurotransmitters, the enzymes of crucial metabolic or regulatory pathways (*e.g.*, dihydrofolate reductase, acetylcholinesterase), proteins involved in transport processes (*e.g.*, Na^+,K^+-ATPase), or proteins that serve structural roles (*e.g.*, tubulin). Specific binding properties of other cellular constituents also can be exploited. Thus, nucleic acids are important drug receptors, particularly for cancer chemotherapeutic agents.

A particularly important group of drug receptors are proteins that normally serve as receptors for endogenous regulatory ligands (*e.g.*, hormones, neurotransmitters). Many drugs act on such physiological receptors and are often particularly selective, because physiological receptors are specialized to recognize and respond to individual signal-

ing molecules with great selectivity. Drugs that bind to physiological receptors and mimic the effects of the endogenous regulatory compounds are termed *agonists.* Other drugs bind to receptors and do not mimic, but interfere with, the binding of the endogenous agonist. Such compounds, which are themselves devoid of intrinsic regulatory activity, but which produce effects by inhibiting the action of an agonist (*e.g.,* by competition for agonist binding sites), are termed *antagonists.* There are additional subtleties to drug classification. Thus, agents that are only partly as effective as agonists are termed *partial agonists,* and those that stabilize the receptor from undergoing productive agonist-independent conformational changes are termed *negative antagonists* or *inverse agonists.* (*See* below, "Quantitation of Drug–Receptor Interactions and Elicited Response.")

The binding of drugs to receptors can involve all known types of interactions—ionic, hydrogen bonding, hydrophobic, van der Waals, and covalent. In most interactions between drugs and receptors, it is likely that bonds of multiple types are important. If binding is covalent, the duration of drug action is frequently, but not necessarily, prolonged. Noncovalent interactions of high affinity also may appear to be essentially irreversible.

Structure–Activity Relationship and Drug Design. Both the affinity of a drug for its receptor and its intrinsic activity are determined by its chemical structure. This relationship is frequently quite stringent. Relatively minor modifications in the drug molecule may result in major changes in pharmacological properties.

Exploitation of structure–activity relationships has on many occasions led to the synthesis of valuable therapeutic agents. Because changes in molecular configuration need not alter all actions and effects of a drug equally, it is sometimes possible to develop a congener with a more favorable ratio of therapeutic to toxic effects, enhanced selectivity among different cells or tissues, or more acceptable secondary characteristics than those of the parent drug. Therapeutically useful antagonists of hormones or neurotransmitters have been developed by chemical modification of the structure of the physiological agonist. Minor modifications of structure also can have profound effects on the pharmacokinetic properties of drugs.

Given adequate information about both the molecular structures and the pharmacological activities of a relatively large group of congeners, it should be possible to identify those properties that are required for optimal action at the receptor—size, shape, the position and orientation of charged groups or hydrogen bond donors, and so on. Recent advances in computational chemistry, structural analysis of organic compounds, and the biochemical measurement of the primary actions of drugs at their receptors have enriched the quantitation of structure–activity relationships and its use in drug design (Kuntz, 1992; Schreiber, 1992). By accurately and quantitatively correlating the pharmacological activities of multiple drugs with their molecular structures—their overall shapes and the locations and orientations of chemically interactive groups on their surfaces—it is possible to model accurately the structure of the binding site on the receptor. Such detailed models allow the informed design of improved congeners or the *de novo* design of novel compounds that can bind to the receptor with improved selectivity, affinity, or regulatory effect. Such considerations also allow computerized searching of large chemical libraries for diverse compounds that, because of their overall three-dimensional structures, should act upon the receptor of interest. Similar structure-based approaches also can be used to improve pharmacokinetic properties of drugs (*see* Chapter 1).

Recent advances using the structures of receptors and of drug-receptor complexes, determined at atomic resolution by X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy, are even more helpful in the initial design of ligands. In cases where the structure of the entire receptor is unknown, it is often possible to determine the conformation of the bound drug, thereby providing a mirror image of the receptor's binding site. The ability to clone and express cDNAs that encode less abundant regulatory proteins and increasing success in the crystallization of membrane-bound proteins offer great promise for drug design based on a detailed knowledge of the drug binding site and the effect of drug binding on receptor structure.

Ironically, advances in molecular biology that contribute to structure-motivated drug design also have spawned powerful but entirely random searches for new drugs. In this approach, huge libraries of randomly synthesized chemicals are generated either by synthetic chemistry or by genetically engineered microbes. A library then is screened for pharmacologically active agents using mammalian cells or microorganisms that have been engineered to express the receptor of therapeutic interest and the associated biochemical machinery necessary for detection of the receptor's response. Active compounds initially discovered by such random screens then can be modified and improved using knowledge of their structure–function relationships.

Cellular Sites of Drug Action. The sites at which a drug acts and the extent of its action are determined by the localization and functional capacity of the specific receptors with which the drug interacts and the concentration of drug to which the receptor is exposed. Selective localization of drug action within the organism is therefore not necessarily dependent upon selective distribution of the drug.

If a drug acts on a receptor that serves functions common to most cells, its effects will be widespread. If the function is a vital one, the drug will be particularly difficult or dangerous to use. Nevertheless, such a drug may be clinically important. Digitalis glycosides, important in the treatment of heart failure, are potent inhibitors of an ion transport process that is vital to most cells. As such, they can cause widespread toxicity, and their margin of safety is dangerously low. Other examples could be cited, particularly in the area of cancer chemotherapy.

If a drug interacts with receptors that are unique to only a few types of differentiated cells, its effects are more specific. Hypothetically, the ideal drug would cause its therapeutic effect by such an action. Side effects would be minimized, but toxicity might not be. If the differentiated function were a vital one, this type of drug also could be very dangerous. Some of the most lethal chemical agents known (*e.g.,* botulinus toxin) show such specificity and toxicity. Note

also that, even if the primary action of a drug is localized, the physiological effects of the drug may be widespread.

Receptors For Physiological Regulatory Molecules

The term *receptor* has been used operationally to denote any cellular macromolecule to which a drug binds to initiate its effects. Among the most important drug receptors are cellular proteins whose normal function is to act as receptors for endogenous regulatory ligands—particularly hormones, growth factors, neurotransmitters, and autacoids. The function of such physiological receptors consists of binding the appropriate ligand and, in response, propagating its regulatory signal in the target cell.

Identification of the two functions of a receptor, ligand binding and message propagation, led to speculation on the existence of functional domains within the receptor: a *ligand-binding domain* and an *effector domain.* The evolution of different receptors for diverse ligands that act by similar biochemical mechanisms, on the one hand, and of multiple receptors for a single ligand that act by unrelated mechanisms, on the other, supports this concept. Indeed, elucidation of the structure of a well-characterized receptor often allows the identification of these specialized domains within the primary amino acid sequence or the three-dimensional structure of the protein.

The regulatory actions of a receptor may be exerted directly on its cellular target(s), *effector protein(s),* or may be conveyed to cellular targets by intermediary cellular molecules, *transducers.* The receptor, its cellular target, and any intermediary molecules are referred to as a *receptor–effector system* or *signal transduction pathway.* Even the effector protein may not be the ultimate cellular component affected but may synthesize or release another signaling molecule, usually a small metabolite or ion, known as a *second messenger.*

Receptors (and their associated effector and transducer proteins) also act as integrators of extracellular information as they coordinate signals from multiple ligands with each other and with the metabolic activities of the cell (*see* below). This integrative function is particularly evident when one considers that the different receptors for scores of chemically unrelated ligands utilize relatively few biochemical mechanisms to exert their regulatory functions and that even these few pathways may share common elements.

An important property of physiological receptors, which also makes them excellent targets for drugs, is that they act catalytically and hence are biochemical signal amplifiers. The catalytic nature of receptors is obvious when the receptor itself is an enzyme, but all known physiological receptors are formally catalysts. For example, when a single ligand molecule binds to a receptor that is an ion channel and opens it, many ions flow through the channel. Similarly, a single steroid hormone molecule binds to its receptor and initiates the transcription of many copies of specific mRNAs, which in turn can give rise to multiple copies of a single protein.

Physiological Receptors: Structural and Functional Families. The last decade has witnessed both an explosion in our appreciation of the number of physiological receptors and, in parallel, the development of our understanding of the fundamental structural motifs and biochemical mechanisms that characterize them. Molecular cloning has identified both completely novel receptors (and their regulatory ligands) and numerous isoforms of previously known receptors. There now exist data banks devoted exclusively to structures of a single class of receptors. Members of various classes of receptors and many of the associated transducer and effector proteins have been purified, and their mechanisms of action are understood in considerable biochemical detail. Receptors, transducers, and effectors can be expressed via molecular genetic strategies and studied in cultured cells. Alternatively, they can be expressed in large amounts in cells of convenience (bacteria, yeast, *etc.*) to facilitate their purification.

Receptors for physiological regulatory molecules can be assigned to several functional families whose members share both common mechanisms of action and, with the exception of the protein kinases, strikingly homologous structures. This homology and the results of extensive biochemical and genetic studies have advanced the two-domain concept of receptor structure beyond conjecture. For each receptor family, there is now at least a rudimentary understanding of the structures of ligand-binding domains and effector domains and of how agonist binding influences the regulatory activity of the receptor. The small number of mechanisms and structural formats has profound implications for both the integration of signals from receptors for diverse ligands and the regulation of receptors and receptor–effector systems by the target cell. It also facilitates a clear overview of signaling pathways that is generally applicable to diverse tissues, disease states, and therapeutic agents. Figure 2–1 provides a schematic diagram of various receptor families and their transducer and effector molecules.

Receptors as Enzymes: Receptor Protein Kinases. Receptors for peptide hormones that regulate growth, differentiation, and development (and in some cases acute metabolic activity) are frequently plasma membrane-bound protein kinases that act by phosphorylating

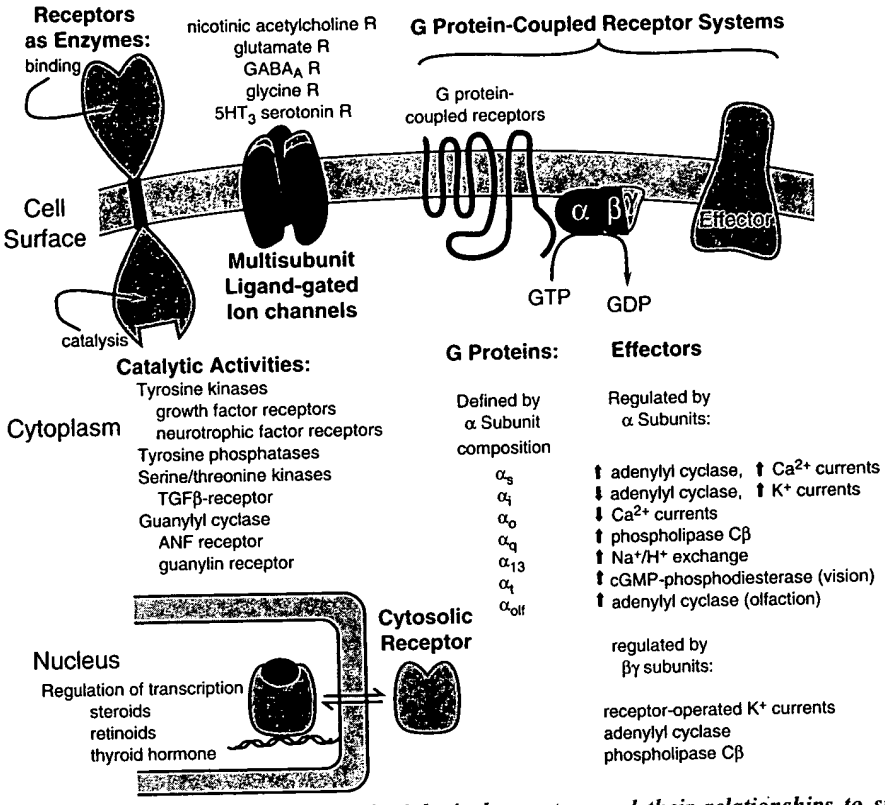

Figure 2–1. Structural motifs of physiological receptors and their relationships to signaling pathways.

Schematic diagram of the diversity of mechanisms for control of cell function by receptors for endogenous agents acting via the cell surface or in the nucleus.

target proteins (Fantl *et al.*, 1993). These targets may be enzymes (including other kinases), regulatory proteins, or structural proteins, and phosphorylation may either alter their individual activities or influence their interactions with other regulatory proteins or effectors. Many receptor protein kinases phosphorylate specific tyrosine residues on their target proteins, but a few phosphorylate serine or threonine residues. Receptors that are tyrosine protein kinases include those receptors for insulin, epidermal growth factor, platelet-derived growth factor, and certain lymphokines. Receptors that are serine/threonine protein kinases include the isoforms of receptors for transforming growth factor β. These receptor kinases are assembled from definable domains that are distinguished in part by their location relative to the plasma membrane. The extracellular, hormone-binding domain is connected to an intracellular protein kinase catalytic domain by a relatively short sequence of hydrophobic amino acid residues that cross the plasma membrane; some members of the receptor kinase families are monomers, and others are assembled from nonidentical subunits. Because of the homology among the protein kinase domains in this family, active chimeric receptors have been constructed from different intracellular (catalytic) and extracellular (hormone binding) regions; these chimeras display specificities for hormones and substrates that reflect their parentage. A substantial literature suggests that oligomerization of tyrosine kinase receptors is a critical event in their activation and alteration of cellular functions.

Another family of receptors that are functionally protein kinases contains a modification of the structures described above. Some protein kinase–associated receptors lack the intracellular enzymatic domains but, in response to agonists, bind and/or activate independent membrane-embedded or cytosolic protein kinases. Receptors of this family that elicit tyrosine phosphorylation include several receptors for neurotrophic peptides and the multisubunit antigen receptors on T and B lymphocytes. There is evidence that the antigen receptors also involve tyrosine protein phosphatases in their cellular regulatory activity, and it is plausible that other receptors that apparently lack cytoplasmic effector domains may recruit still other effector proteins.

Receptors with Other Enzymatic Activity. The domain structure just described for cell surface protein kinases is varied in other receptors to utilize other signaling outputs. In the receptors for atrial natriuretic peptides and the peptide guanylin, the intracellular domain is not a protein kinase but is a guanylyl cyclase, which synthesizes the second messenger, cyclic GMP (Chinkers and Garbers, 1991). Receptors with guanylyl cyclase activity also serve as pheromone receptors in invertebrates. There may be other variations on this transmembrane topology.

A family of protein tyrosine phosphatases has extracellular domains with a sequence reminiscent of cellular adhesion molecules (Walton and Dixon, 1993). Although the extracellular ligands for these phosphatases are not yet known, the importance of their enzymatic activity has been demonstrated through genetic and biochemical experiments in multiple cell types.

Ion Channels. Receptors for several neurotransmitters form agonist-regulated, ion-selective channels in the plasma membrane, termed *ligand-gated ion channels,* which convey their signals by

altering the cell's membrane potential or ionic composition (Hall, 1992). This group includes the nicotinic cholinergic receptor, the $GABA_A$ receptor for gamma-aminobutyric acid, and receptors for glutamate, aspartate, and glycine (*see* Chapters 7, 9, and 12). They are all multi-subunit proteins with each subunit predicted to span the plasma membrane. The mode of association of the subunits appears to form the channel. Of therapeutic importance is that allosteric modifiers of channel gating can have profound pharmacological effects; for example, the benzodiazepines allosterically enhance Cl^- transport through the $GABA_A$ receptor (*see* Chapters 17 and 18).

G Protein–Coupled Receptors. Many receptors in the plasma membrane regulate distinct effector proteins through the mediation of a group of GTP-binding proteins known as G proteins (Ross, 1992). Receptors for biogenic amines, eicosanoids, and many peptide hormones utilize G protein–coupled receptors. Receptors in this group act by facilitating the binding of GTP to specific G proteins. GTP binding activates the G protein, such that it in turn can regulate the activity of specific effectors. The effectors include enzymes such as adenylyl cyclase and phospholipases A_2, C, and D; channels that are specific for Ca^{2+}, K^+, or Na^+; and certain transport proteins. An individual cell may express multiple G proteins; each of these may respond to several different receptors and regulate several different effectors with a characteristic pattern of selectivities. G protein–linked receptors and the G proteins themselves both constitute families of homologous proteins. The receptors are hydrophobic proteins that span the plasma membrane in seven α-helical segments. The binding site for small ligands can be a pocket within the bundle of membrane-spanning helices, but a substantial extracellular domain is important for the binding of negatively charged ligands, such as glutamate, or of peptide hormones. The receptors interact with G proteins at their cytoplasmic face, and it has been possible to define specific regions in G protein–coupled receptor structures that are responsible for regulation of and selectivity among the different G proteins.

The G proteins are bound to the inner face of the plasma membrane. They are heterotrimeric molecules (subunits are designated α, β, and γ), and their classification is based on the identity of their distinct α subunits. These polypeptides have highly homologous guanine nucleotide binding domains and have distinct domains for interactions with receptors and effectors. When the system is inactive, GDP is bound to the α subunit (Figure 2–2). An agonist–receptor complex facilitates GTP binding to the α subunit in part by promoting the dissociation of bound GDP. Binding of GTP activates the α subunit, and the α-GTP subunit is then thought to dissociate from the $\beta\gamma$ subunits and interact with a membrane-bound effector. The $\beta\gamma$ subunits also can interact with and influence effector activity independent of or in parallel with α-GTP subunit effects (Clapham and Neer, 1993). Termination of signal transmission results from hydrolysis of GTP to GDP by a GTPase that is intrinsic to the α subunit and the resulting reassociation of α and $\beta\gamma$ subunits. Thus, G proteins serve as regulated molecular switches capable of eliciting bifurcating signals through α and $\beta\gamma$ subunit effects. The switch is turned on by the receptor and turns itself off within a few seconds, a time sufficient for considerable amplification of signal transmission.

If a cell has several receptors that regulate a common effector or that utilize a common transducer, many individual extracellular signals can be integrated to yield a cumulative intracellular signal. G protein–coupled receptor–effector systems provide impressive examples of such integration, as well as the ability to direct a signal to divergent cellular effectors (*see* Figure 2–3 and related discussion later in this chapter). It is not unusual for several receptors in an individual cell to activate a single G protein; several agonists may stimulate adenylyl cyclase through a single G protein known as G_s. One receptor also can regulate more than one G protein; an individual thrombin receptor can cause the inhibition of adenylyl cyclase and the activation of phospholipase C by interactions with at least two different G proteins. Similarly, one G protein can regulate several effectors. Thus, the receptor-G protein–effector systems are complex networks of convergent and divergent interactions that permit extraordinarily versatile regulation of cell function (Ross, 1992).

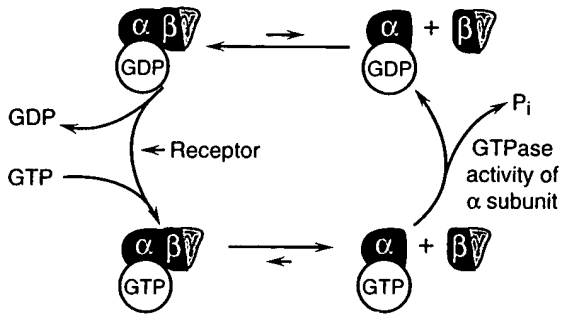

Figure 2–2. The regulatory cycles involved in G protein–mediated signal transduction.

GTP-binding protein activation of effectors is regulated simultaneously by a GTPase cycle and subunit association/dissociation cycle. The GTP-liganded α subunit activates some processes exclusively, and release of $\beta\gamma$ subunits upon activation of G_α allows for regulation by $\beta\gamma$ subunits of shared or distinct effectors.

Transcription Factors. Receptors for steroid hormones, thyroid hormone, vitamin D, and the retinoids are soluble DNA-binding proteins that regulate the transcription of specific genes (Evans, 1988; Mangelsdorf *et al.*, 1994). They are part of a larger family of transcription factors whose members may be regulated by phosphorylation, association with other protein factors, or by binding to metabolites or cellular regulatory ligands. These receptors act as dimers, some as homodimers and some as heterodimers, with homologous cellular proteins, but may be regulated by higher order oligomerization with other regulating molecules. They provide striking examples of conservation of structure and mechanism, in part because they are assembled as three largely independent domains. The region nearest the carboxyl terminus binds hormone and serves a negative regulatory role; that is, removal of this domain leaves a constitutively active fragment that may be nearly as effective in regulating transcription as is the intact hormone-liganded receptor. Hormone binding presumably also relieves this inhibitory constraint. The central region of the receptor mediates binding to specific sites on nuclear DNA to activate or inhibit transcription of the nearby gene. These regulatory sites in DNA are likewise receptor-specific: the sequence of a "glucocorticoid-responsive element," with only slight variation, is associated with each glucocorticoid-responsive gene. The function of the amino-terminal region of the receptor is less well defined, but its loss decreases the receptor's regulatory activity. The DNA-binding receptors form a homologous family, sharing quite similar amino acid sequences in their DNA-binding domains, less similarity in their hormone-binding domains, and negligible similarity at their amino termini. The activity of each domain is stereotyped and largely independent, a phenomenon best demonstrated by the construction of chimeric receptors that reflect the hormone binding or DNA regula-

tory activity characteristic of the "parent" receptor contributing that domain.

Cytoplasmic Second Messengers. Physiological signals also are integrated within the cell as a result of interactions between second messenger pathways. There are relatively few recognized cytoplasmic second messengers. Thus, their synthesis or release reflects the activities of many pathways. Second messengers influence each other both directly, by altering the other's metabolism, and indirectly, by sharing intracellular targets. This superficially confusing pattern of regulatory pathways allows the cell to respond to agonists, singly or in combination, with an integrated array of cytoplasmic second messengers and responses (*see* Figure 2–3).

Cyclic AMP, the first recognized second messenger, is synthesized by adenylyl cyclase in response to activation of many receptors; stimulation is mediated by G_s and inhibition by one or more closely related G proteins termed G_i's. There exist at least ten tissue-specific adenylyl cyclase isozymes, each with its unique pattern of regulatory responses (Taussig *et al.*, 1994). Several adenylyl cyclase isozymes are inhibited by the G protein $\beta\gamma$ subunits, which allows activation of G proteins other than G_s to inhibit cyclase activity. Other isozymes are stimulated by $G\beta\gamma$ subunits, but this stimulation is dependent upon concurrent stimulation by the α subunit of G_s. Still other isozymes are stimulated by Ca^{2+} or Ca^{2+}–calmodulin complexes. Finally, each of the isozymes has its own pattern of enhancement or attenuation by phosphorylation or other regulatory influences, providing a broad array of regulatory features to the target cells where these isoforms are expressed.

The hydrolysis of cyclic AMP is catalyzed by several phosphodiesterases (Strada and Hidaka, 1992), and the extrusion of cyclic AMP from the cell is accomplished by at least one regulated active transport system. In most cases, cyclic AMP functions by activating cyclic AMP-dependent protein kinases, which regulate numerous intracellular proteins by catalyzing their phosphorylation (*see* Edelman *et al.*, 1987). In at least one case, olfaction, cyclic AMP mediates signaling by direct binding to and allosteric activation of ligand-activated Na^+ channels.

The cytoplasmic concentration of Ca^{2+}, another ubiquitous second messenger, is controlled both by regulation of several different Ca^{2+}-specific channels in the plasma membrane and by its release from intracellular storage sites. Ca^{2+} channels can be opened by electrical depolarization, by phosphorylation by a cyclic AMP-dependent protein kinase, by G_s, by K^+, or by Ca^{2+} itself. Opening can be inhibited by other G proteins (G_i and G_o). One channel may respond to several of these inputs.

Release of Ca^{2+} from intracellular stores is mediated by yet another second messenger, inositol 1,4,5-trisphosphate (IP_3). IP_3 is the product of the hydrolysis of the membrane lipid, phosphatidylinositol 4,5-bisphosphate (PIP_2); this reaction is catalyzed by a phospholipase C (PLC; *see* Figure 2–3). Three families of PLCs, each with several homologous members, respond to three distinct signaling pathways. The PLC-β's (there is no PLC-α) are stimulated

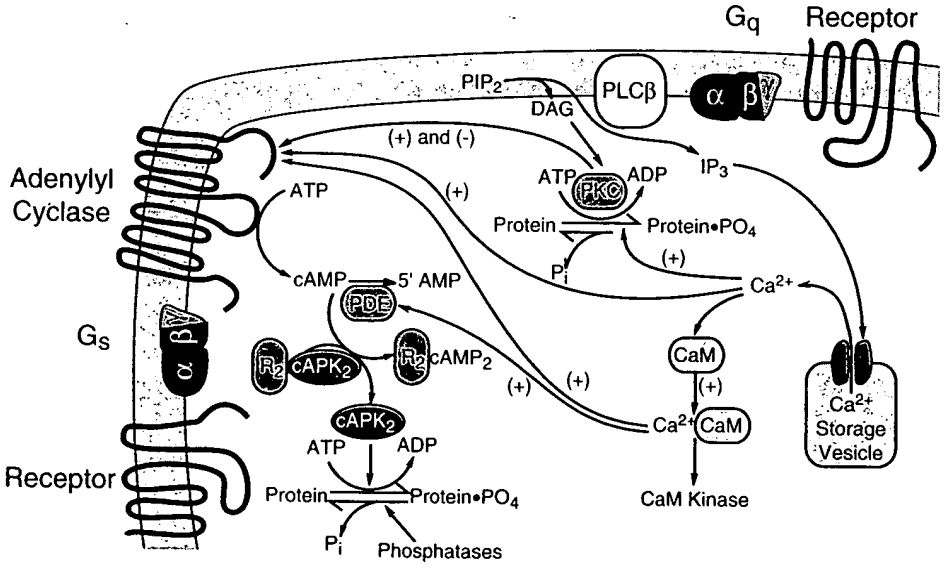

Figure 2–3. Interactions between the second messengers cyclic AMP and Ca^{2+}.

Generation of *second messengers*, cyclic AMP (cAMP) and Ca^{2+}, permits distribution of cell-surface regulatory input within the cell interior, amplification of the initial signal, and opportunities for synergistic or antagonistic regulation of other signaling pathways. PIP_2, phosphatidyl inositol-4,5, bisphosphate; DAG, diacylglycerol; IP_3, 1,4,5 inositol trisphosphate; CaM, calmodulin; R_2, regulatory subunits of cyclic AMP-dependent protein kinase, which bind cyclic AMP; $cAPK_2$, catalytic subunits of cyclic AMP-dependent protein kinase; PKC, protein kinase C, activated by DAG and Ca^{2+}.

by the G_q family of G proteins and, for some members, by the G protein $\beta\gamma$ subunits. The PLC-γ's are activated by phosphorylation on tyrosine residues and are thus activated by cell surface receptor-activated tyrosine kinase cascades. It is not known what regulates the PLC-δ's (Rhee and Choi, 1992).

Ca^{2+} regulates cellular activity by interaction with several protein mediators; salient examples are protein kinase C and calmodulin (Berridge, 1993). Protein kinase C, like the cyclic AMP-dependent protein kinase, has many substrates, including several proteins that are involved in other signaling systems. The activation of protein kinase C by Ca^{2+} is potentiated by diacylglycerol, the other second messenger product of the phospholipase C-catalyzed reaction that liberates IP_3. The scope of calmodulin's regulatory activity also is broad. Thus, with reference only to the examples of cyclic AMP and Ca^{2+}, one can appreciate the complexity of integration of cellular signaling systems as reflected in Figure 2–3.

Regulation of Receptors

It is important to recognize that receptors not only initiate regulation of physiological and biochemical function but also are themselves subject to many regulatory and homeostatic controls. For example, continued stimulation of cells with agonists generally results in a state of *desensitization* (also referred to as *refractoriness* or *down regulation*), such that the effect that follows continued or subsequent exposure to the same concentration of drug is diminished (Figure 2–4). This phenomenon can become very important in therapeutic situations; an example is attenuated response to the repeated use of β-adrenergic agonists as bronchodilators for the treatment of asthma (*see* Chapter 10).

Multiple mechanisms account for desensitization of different types (*see* Perkins *et al.*, 1990). In some cases, only the signal from the stimulated receptor becomes attenuated, a process known as *homologous desensitization*. This may involve covalent modification (*e.g.*, phosphorylation) of the receptor, the destruction of the receptor, or its relocalization within the cell. Synthesis of receptors also is subject to feedback regulation. In other situations, receptors for different hormones that act on a single signaling pathway may all become less effective when only one is continuously stimulated. Such *heterologous desensitization* may result either from modification of each receptor by a common feedback mechanism or from effects exerted at some common point in the effector pathway distal to the receptor itself. Mechanisms involved in homologous and heterologous desensitization of specific receptors and signaling pathways will be discussed in greater detail in later chapters related to individual receptor families.

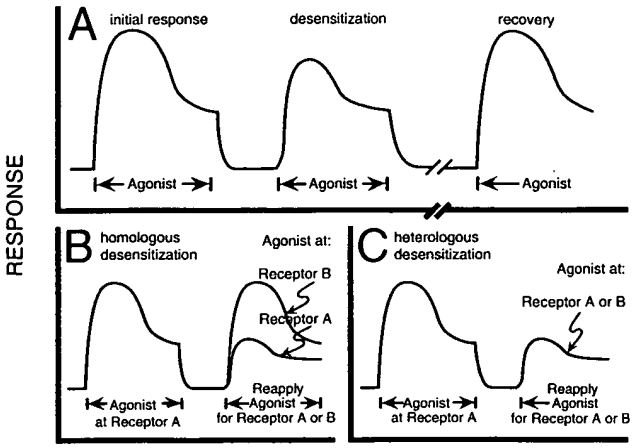

Figure 2–4. Desensitization in response to an agonist.

A. Upon exposure to an agonist, the *initial response* usually peaks and then decreases to approach some tonic level, elevated but below the maximum. If the drug is removed for a brief period, the state of *desensitization* is maintained such that a second addition of agonist also provokes a diminished response. Removal of the drug for a more extended period allows the cell to "reset" its capacity to respond, and *recovery* of response usually is complete. (***B*** and ***C***) Desensitization may be *homologous* (***B***), affecting responses elicited only by the stimulated receptor, or *heterologous* (***C***), acting on several receptors or on a pathway that is common to many receptors.

Predictably, hyperreactivity or supersensitivity to receptor agonists also frequently is observed to follow reduction in the chronic level of receptor stimulation. Situations of this type can result, for example, from the long-term administration of β-adrenergic antagonists such as propranolol (*see* Chapter 10). In at least some cases, supersensitivity may result from the synthesis of additional receptors.

Diseases Resulting from Receptor Malfunction. In addition to variability among individuals in their responses to drugs (*see* Chapter 3), several definable diseases arise from disorders in receptors or receptor–effector systems. The loss of a receptor in a highly specialized signaling system may cause a relatively limited phenotypic disorder, such as the genetic deficiency of the androgen receptor in the testicular feminization syndrome (Griffin and Wilson, 1989). Deficiencies of more widely used signaling systems have a broader spectrum of effects, as are seen in myasthenia gravis or some forms of insulin-resistant diabetes mellitus, which result from autoimmune depletion of nicotinic cholinergic receptors (*see* Chapter 9) or insulin receptors (*see* Chapter 60), respectively. A lesion in a component of a signaling pathway that is used by many receptors can cause a generalized endocrinopathy. Heterozygous deficiency of G_s, the G pro-

tein that activates adenylyl cyclase in all cells, causes multiple endocrine disorders; the disease is termed *pseudohypoparathyroidism type Ia* (Spiegel, 1989). Homozygous deficiency in G_s would presumably be lethal.

The expression of aberrant or ectopic receptors, effectors, or coupling proteins potentially can lead to supersensitivity, subsensitivity, or other untoward responses. Among the most interesting and significant events is the appearance of aberrant receptors as products of *oncogenes*, which transform otherwise normal cells into malignant cells. Virtually any type of signaling system may have oncogenic potential. The *erb*A oncogene product is an altered form of a receptor for thyroid hormone, constitutively active because of the loss of its ligand-binding domain (Evans, 1988). The *ros* and *erb*B oncogene products are activated, uncontrolled forms of the receptors for insulin and epidermal growth factor, both known to enhance cellular proliferation (Yarden and Ullrich, 1988). The *mas* oncogene product (Young *et al.*, 1986) is a G protein–coupled receptor, probably the receptor for a peptide hormone. Constitutive activation of G protein–coupled receptors due to subtle mutations in receptor structure has been shown to give rise to retinitis pigmentosa, precocious puberty, and malignant hyperthyroidism (reviewed in Clapham, 1993). G proteins can themselves be oncogenic when either overexpressed or constitutively activated by mutation (Lyons *et al.*, 1990).

Detection and Characterization of Receptors by Ligand-Binding Assays. Much of the success in the identification, purification, and characterization of receptors reflects their quantitative measurement according to the binding of highly specific radioactive ligands (Limbird, 1995). Binding assays permit the direct study of the drug-binding properties of receptors and reduce the need to rely on inferences derived from the measurement of distal physiological responses. Such inferences are confounded if the observed response lies many steps removed from the receptor and may be compromised by changes at any site in the pathway leading from receptor to ultimate effector. Direct measurement of receptors and analysis of their ligand-binding properties and mechanisms of action have led to our understanding of the mechanisms of receptor action, pathophysiology, and therapeutic effects. For example, we now appreciate the molecular causes of many diseases of receptor malfunction (*see* above).

Classification of Receptors and Drug Effects

Traditionally, drug receptors have been identified and classified primarily on the basis of the effect and relative potency of selective agonists and antagonists. For example, the effects of acetylcholine that are mimicked by the alkaloid muscarine and that are selectively antagonized by atropine are termed *muscarinic effects*. Other effects of acetylcholine that are mimicked by nicotine and that are not readily antagonized by atropine but are selectively blocked by other agents (*e.g.*, tubocurarine) are described as *nicotinic effects*. By extension, these two types of cholinergic effects are said to be mediated by muscarinic or nicotinic receptors. Such classification of receptors results in an internally consistent scheme that supports the view that two types of receptor are involved. Although it frequently contributes little to delineation of the mechanism of drug action, such categorization provides a convenient basis for summarizing drug effects. A statement that a drug activates a specified type of receptor is a succinct summary of its spectrum of effects and of the agents that will antagonize it. Similarly, a statement that a drug blocks a certain type of receptor specifies the agents that it will antagonize and at what sites. However, it should be appreciated that the accuracy of this statement may be altered when additional receptor subtypes are identified or additional drug mechanisms or side effects are revealed.

Significance of Receptor Subtypes. As the diversity and selectivity of drugs have increased, it has become clear that multiple subtypes of receptors exist within many previously defined classes of receptors. Moreover, molecular cloning frequently has revealed the presence of several closely related subtypes of receptors where only a single species was thought to exist, and some receptor subtypes have been shown to be differentially expressed during development. Knowledge of receptor subtypes is of interest to the researcher and of utility to the clinician who desires to manipulate them.

In the case of the nicotinic cholinergic receptor, referred to above, there are distinct differences in the ligand-binding and functional properties between the receptors that are found in the ganglia of the autonomic nervous system and those at the somatic neuromuscular junction. This difference is exploited for therapeutic benefit. Thus, antagonists that act preferentially at the nicotinic receptors in ganglia can be used to control blood pressure without paralyzing skeletal muscle. Tubocurarine and related agents constitute the converse example; their ability to antagonize the action of acetylcholine is relatively well confined to the receptor sites at the neuromuscular junction (*see* Chapter 9). These subtypes of the nicotinic receptor as well as subtypes of, for example, β-adrenergic receptors (*see* Chapter 10) are conceptually analogous to tissue-specific isozymes of an enzyme.

Although the mechanisms of action of some receptor subtypes may be very similar, differing only slightly in kinetics or regulatory activity, other receptor subtypes display fundamental differences in their biochemical and cellular regulatory activities. Examples include the α_1- and α_2-adrenergic receptors and the M_1- and M_2-muscarinic cholinergic receptors. Although all four receptor subtypes regulate G proteins, the α_1-adrenergic and the M_1- (and M_3-) muscarinic receptors initiate Ca^{2+} signaling *via* G_q, whereas the α_2-adrenergic and the M_2- (and M_4-) muscarinic receptors regulate other signaling pathways *via* G_i and another GTP binding protein, G_o (*see* Chapters 7 and 10). Expression of such different receptor subtypes allows a single agonist to evoke unique responses in specific cells or tissues.

When a tissue or cell expresses more than a single subtype of receptor or when only insufficiently selective drugs are available, identification of the specific signal that is generated by an individual receptor requires more direct approaches. These include the expression of the cloned cDNA for the receptor in a well-characterized cell where its signaling activities can be studied in detail, the expression and purification of the recombinant receptor for direct biochemical

analysis of its functions, or the use of antisense strategies to evaluate which signal-transducing pathway is necessary for agonist effects.

The discovery by molecular cloning of numerous receptor subtypes raises the question of their importance, particularly when their signaling mechanisms and specificity for endogenous ligands are indistinguishable. Perhaps the multiplicity of genes facilitates their independent, cell-specific and developmentally controlled expression, allowing receptor subtypes to be expressed independently according to the developmental needs of the organism. Regardless of their mechanistic implications (or lack thereof), schemes of receptor classification have facilitated the development of a number of therapeutic agents that have selectivity for specific types or subtypes of receptors. Such drug development has provided the clinician with compounds having higher ratios of therapeutic effects to toxic or unwanted effects.

Actions of Drugs Not Mediated By Receptors

If one restricts the definition of receptors to macromolecules, then several drugs may be said not to act by virtue of combination with receptors. Certain drugs interact specifically with small molecules or ions that are normally or abnormally found in the body. One example is the therapeutic neutralization of gastric acid by a base (antacid). Another example is the use of mesna, a free radical scavenger rapidly eliminated by the kidneys, to bind to reactive metabolites associated with some cancer chemotherapeutic agents and thus minimize their untoward effects on the urinary tract (*see* Chapter 51). Other agents act more by virtue of their colligative effects than by more classical chemical mechanisms. Such mechanisms are characterized by a lack of requirement for highly specific chemical structure. For example, certain relatively benign compounds, such as mannitol, can be administered in quantities sufficient to increase the osmolarity of various body fluids and thereby cause appropriate changes in the distribution of water (*see* Chapter 29). Depending on the agent and route of administration, this effect can be exploited to promote diuresis, catharsis, expansion of circulating volume in the vascular compartment, or reduction of cerebral edema.

Certain drugs that are structural analogs of normal biological chemicals may be incorporated into cellular components and thereby alter their function. This property has been termed a "counterfeit incorporation mechanism," and has been particularly useful with analogs of pyrimidines and purines that can be incorporated into nucleic acids; such drugs have clinical utility in cancer and antiviral chemotherapy (*see* Chapters 50 and 51).

QUANTITATION OF DRUG–RECEPTOR INTERACTIONS AND ELICITED RESPONSE

As early as 1878, even before he coined the term *receptive substance,* Langley suggested that drug–cell combinations, and hence the actions and effects of drugs, were probably governed by the law of mass action. This view was developed further by A. J. Clark in the 1920s (*see* monograph published in 1933), and it remains basic to understanding the mechanisms of drug action.

Extending the analysis of drug–receptor interactions beyond the initial binding of drug to receptor raises important questions as to the relationship between the concentration of drug–receptor complex and the magnitude of the observed effect. In the classical receptor theory developed by Clark, it was assumed that the effect of a drug is proportional to the fraction of receptors occupied by drug and that maximal effect results when all receptors are occupied. While these assumptions may be true in limited cases, exceptions are common. Consequently, Ariens (1954) introduced the term *intrinsic activity* (or α, a "proportionality constant") to describe the relationship between the effect, E, elicited by a drug, D, and the concentration of drug–receptor complexes: $E = \alpha[DR]$. This relationship also addressed the anomalous observation that some drugs did not elicit a maximal response even at apparently maximal receptor occupancy. It was Stephenson (1956), however, who advanced the concept of concentration–response relationships even further by offering an explanation for nonlinear relationships between occupancy and response and for the ability of a group of agonists to produce equal responses while occupying different proportions of the receptor population. This explanation involves a property of an agonist that Stephenson called *efficacy.* In the terms of Stephenson, the response, R, of a tissue is some function of the stimulus, S, given to that tissue: $R = f(S)$ and $S = ey$, where e = efficacy and y = fractional receptor occupancy. Differences in drug effects in different tissues are thus a reflection of the contributions of properties of the drug, properties of its receptor, and properties of a tissue in terms of receptor density and the coupling of receptor occupancy to the ultimate response. Today, the terms *intrinsic activity* and *efficacy* are commonly used interchangeably and are operationally synonymous. Although the mathematical description of dose–response relationships cannot reveal molecular mechanisms, the basic descriptive concepts are instructive and provide an appreciation of how the concentration of a drug at its target organ determines the therapeutic response.

If one assumes that an agonist drug interacts reversibly with its receptor, that the resultant effect is proportional to the number of receptors occupied, and that a maximum effect occurs when all receptors are occupied, as in the original model of A. J. Clark, the following re-

action scheme can be written:

$$\text{Drug } (D) + \text{receptor } (R) \underset{k_2}{\overset{k_1}{\rightleftharpoons}} DR \rightarrow \text{effect} \qquad (2\text{–}1)$$

The relationship between effect and the concentration of free drug can be described simply for this model as:

$$\text{Effect} = \frac{\text{maximal effect} \cdot [D]}{K_D + [D]} \qquad (2\text{–}2)$$

where $[D]$ is the concentration of free drug and K_D (equal to k_2/k_1) is the equilibrium dissociation constant for the drug–receptor complex. The fraction of receptors that is occupied by drug is equal to $[D]/(K_D + [D])$. This equation describes a simple rectangular hyperbola and is analogous to the Michaelis–Menten equation that is used to describe the interaction of enzyme and substrate where no product is formed (Figure 2–5, *A*). The scheme defines the drug's *potency*—that is, the dependency of effect on its concentration. It is frequently convenient to plot the magnitude of effect versus log $[D]$, because a wide range of drug concentrations is easily displayed and the potency of different drugs can be compared readily. In this case, the result is the familiar sigmoidal log dose–effect curve, probably the most intuitively helpful graphical display of drug action (Figure 2–5, *B*). This representation also allows comparisons of the relative potencies and efficacies of agonists (Figure 2–5, *C*). When there is a linear relationship between occupancy and response, as in the model of A. J. Clark, the concentration of the drug at which it is half-maximally effective, its EC_{50}, is equal to its K_D. However, as noted above, there often is amplification between occupancy and response, such that the EC_{50} for response lies far to the left of the K_D for receptor occupancy (Figure 2–5, *D*).

As stated earlier, *antagonists* bind to the receptor or components of the effector mechanism to inhibit the action of an agonist, but, in the classical definition, initiate no effect themselves. If the inhibition can be overcome by increasing the concentration of the agonist, ultimately achieving the same maximal effect, the antagonist is said to be *competitive* or *surmountable.* This type of inhibition commonly is observed with antagonists that bind reversibly at the receptor site. In the terminology of Stephenson, classical competitive antagonists would have zero efficacy. Because the maximal effect can still be achieved if sufficient agonist is used, the log dose–effect curve for the agonist is shifted to the right by a competitive antagonist (Figure 2–6, *A*). The maximal effect is unaltered, but the agonist appears to be less potent.

The parallel rightward shift in agonist concentration–response curves in the presence of increasing concentration of antagonist (Figure 2–6, *B*) provides an opportunity to further characterize antagonist properties in physiological preparations. Thus, calculation of the ratio of agonist concentrations that elicit equal responses in the absence and presence of antagonist at increasing concentrations (termed the dose ratio) and plotting these values according to the relationship *log*(*dose ratio* − 1) versus *log* [*antagonist*] yields, at the $y = 0$ intercept, the K_D value for antagonist at the receptor (Figure 2–6, *C*). This data transformation, referred to as the Schild regression, provides additional insight into the nature of the antagonist interaction with the receptor. Competitive antagonists interacting with a single

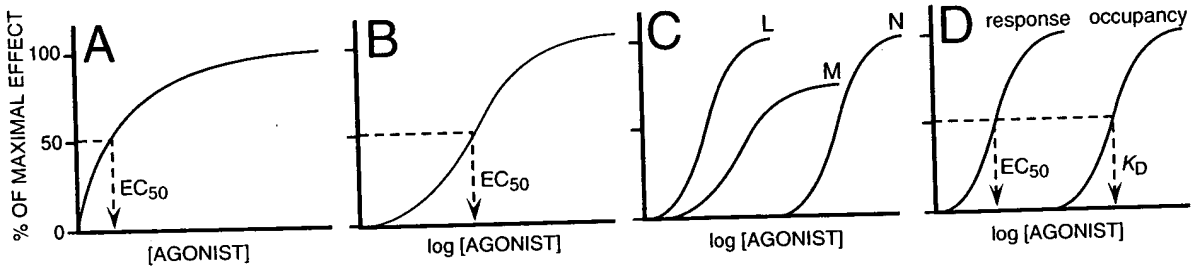

Figure 2–5. Agonist interactions with biological receptors.

A. In the simplest circumstance, agonist occupancy of the receptor obeys the law of mass action, and the relationship between agonist concentration (*linear* scale) and response is reflected by a rectangular hyperbolic relationship. ***B.*** A plot of response versus log [agonist] reveals a sigmoidal relationship between occupancy and response, such that, in the absence of negative or positive cooperativity, 10% to 90% response occurs over approximately a 100-fold range of agonist concentration, "centered" about the EC_{50} for agonist. *C.* Agonists vary in terms of *potency* and *efficacy.* The EC_{50} value represents the concentration of agonist that elicits a half-maximal response. Drug *L* is more potent than drugs *M* and *N*; drugs *L* and *N* are more efficacious than drug *M*, a *partial agonist. D.* Because occupancy often is not directly related to response and signal amplification occurs between receptor occupancy, effector activation, and ultimate response, dose–*response* curves often fall to the left of receptor-*occupancy* profiles.

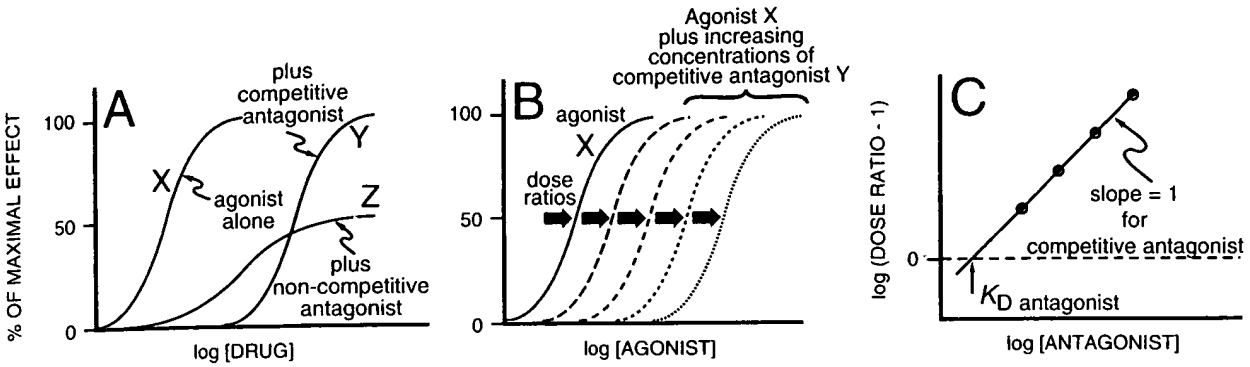

Figure 2–6. Properties of antagonist blockade of response.

A. Log dose–response curves for an agonist in the absence (X) or presence of a competitive (Y) or noncompetitive (Z) antagonist. *B.* Agonist activation of a response in the presence of increasing concentrations of a competitive antagonist yields a series of parallel rightward shifted dose–response curves. The *dose ratio* is the ratio of the agonist concentrations (or doses) required to elicit equal responses in the presence and absence of antagonist. *C.* The Schild regression permits a direct estimate of the K_D for a competitive antagonist for receptor occupancy and hence blockade of response. When the slope of a Schild regression $\neq 1.0$, either the drug is not a competitive antagonist or experimental limitations to interpretation prevail, and the value of x at $y = 0$ has no thermodynamic significance (*see* Kenakin *et al.*, 1992).

population of noninteracting receptors yield a regression line with a slope equal to 1. Antagonist interactions with multiple receptor subtypes having differing affinities for the antagonist or antagonist effects other than those that are strictly competitive and fully reversible yield complex Schild regression plots that are either nonlinear or diverge from a slope of 1 (Schild, 1957; Kenakin *et al.*, 1992). K_D values so derived should be the same when a single antagonist is used with several agonists that act on the same receptor.

A noncompetitive antagonist prevents the agonist, at any concentration, from producing a maximum effect on a given receptor. This could result from irreversible interaction of the antagonist at any site to prevent binding of agonist or from reversible or irreversible interaction with a component of the system so as to decrease or eliminate the effect of the binding of agonist. Intuitively, these results may be conceptualized as removal of receptor or the system's capacity to respond. The maximal effect possible is reduced, but agonist can act normally at receptor–effector units that are not influenced by antagonist. Typically, log dose–effect curves reflect reduced apparent efficacy but unaltered potency in the presence of a noncompetitive antagonist (Figure 2–6, *A*).

Antagonists may be classified as acting reversibly or irreversibly. If the antagonist binds at the active site for the agonist, reversible antagonists will be competitive, and irreversible antagonists will be noncompetitive. If binding is elsewhere, however, these simple rules do not hold, and any combination of functional outcomes is possible.

If two drugs bind to the same receptor at the same site, why can one be an agonist and the other produce no effect—acting as an antagonist? This central question of pharmacodynamics is also central to our understanding of protein structure and protein–ligand interactions. Its answer derives from two active and complementary experimental approaches. On the one hand, structural biophysicists have determined and compared the active and inactive structures of proteins whose activities are controlled by the binding of regulatory ligands. These studies have elucidated the molecular forces that allow one ligand but not another to alter a protein's conformation. Biochemical analysis of protein–ligand interactions, using both enzymatic activity and spectroscopic measures of conformational change, have produced a rich understanding of the energetics and kinetics of allosteric regulation that is consistent with the structural data (Wyman and Gill, 1993; Weber, 1994).

Consider that a receptor must, by definition, exist in at least two conformations: active (a) and inactive (i).

$$\begin{array}{ccc} R_i & \rightleftharpoons & R_a \\ \Updownarrow & & \Updownarrow \\ D \cdot R_i & \rightleftharpoons & D \cdot R_a \end{array}$$

These conformations might correspond to the open and closed states of an ion channel, the active and inactive states of a protein tyrosine kinase, or the productive versus nonproductive conformations of a receptor for coupling to G proteins. The *extent* to which the equilibrium is perturbed is determined by the *relative* affinity of the drug for the two conformations (Figure 2–7). If these states are in equilibrium and the inactive state predominates in the absence of drug, then the basal signal output will be low. In this case, the presence of a drug that has a higher affinity for the active conformation than for the inactive conformation will drive the equilibrium to the active state

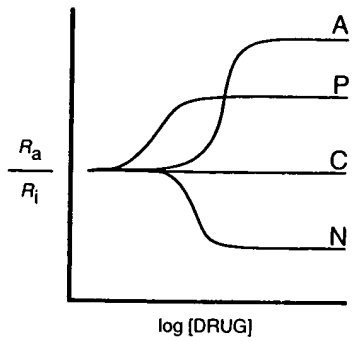

Figure 2–7. A working model for receptor-mediated response.

Effects of drugs on the relative concentrations of two hypothetical forms of a receptor, R_a (active) and R_i (inactive) that are in equilibrium, $R_a \rightleftharpoons R_i$. As discussed in the text, the relative distribution of the receptor between these two forms is differentially influenced by agonists (*A*), partial agonists (*P*), competitive antagonists (*C*), and negative antagonists (*N*), also known as inverse agonists.

and thereby activate the receptor. Such a drug will be an agonist. A full agonist is sufficiently selective for the active conformation so that, at a saturating concentration, it will drive the receptor completely to the active state (*e.g.*, agent *A* in Figure 2–7). If a different but perhaps structurally analogous compound binds to the same site on *R* but with only slightly greater affinity for R_a than for R_i, the magnitude of effect observed may be less, even at saturating concentrations, such as agent *P* in Figure 2–7. A drug that displays such intermediate effectiveness is referred to as a *partial agonist.* Partial agonists are not hypothetical; they are common and were first described quantitatively in the formulations introduced by Ariëns and Stephenson.

A drug that binds with equal affinity to either conformation will not alter the activation equilibrium and will act as a competitive antagonist, shown as drug *C* in Figure 2–6. A partial agonist also can act as an antagonist. When it binds to the receptor (and produces a submaximal response), it also occupies the drug binding site competitively with respect to a full agonist. A greater concentration of a full agonist will be required to produce a maximal effect because of this competition. A drug with preferential affinity for R_i will actually produce an effect opposite to that of an agonist, and examples of so-called *negative antagonists* or *inverse agonists* do exist (*see* Chapters 11 and 17). These agents have properties that are analogous to those of drug *N* in Figure 2–7. However, if the *preexisting* equilibrium lies far in the direction of R_i, negative antagonism may be difficult to observe and to distinguish from simple competitive antagonism. Careful biochemical studies of receptor–drug interactions, coupled with the analysis of receptors in which the intrinsic R_a/R_i equilibrium has been shifted by mutation, have supported this model of drug action. Although the complete mathematical description can be complex (Taylor and Insel, 1990), the model is manageable and readily applicable to experimental data through the use of appropriate computer-assisted analysis. There is, then, the potential ability to design agents that suppress agonist-independent activity of receptors that are hyperactive in the so-called basal state, as opposed to agents that block receptor activity caused by excess availability of agonist.

As stated above, the terms *intrinsic activity* or *efficacy* are used to describe properties of drugs that bind to the same receptor site but do not produce equal maximum effects. Simplistically, the efficacy of a full agonist can be set equal to 1, that of an antagonist to 0, and that of a partial agonist to a value between 0 and 1. Fractional effect is then equal to the product of fractional occupancy of the receptor and the fractional efficacy. As first appreciated by Stephenson, these correction factors are dependent both on the molecular properties of receptors and on the concentrations and interactions of transducer and effector proteins. Thus, these factors are reliable only in a defined and controlled situation; they will change in different tissues and when different responses are measured. Nonetheless, these parameters are of use in evaluating and comparing the therapeutic utility of multiple drugs. Finally, it should be noted that the use of the word *efficacy* can, at times, be confusing. While a competitive antagonist has no efficacy in the sense that it does not serve as an initiator of an action that leads to a sequence of effects, it may have great therapeutic effectiveness, or *clinical efficacy,* when used to block agonist effects in human beings.

Even if the proximal molecular action of an agonist at a receptor is proportional to its efficacy and to the number of receptor sites occupied, the effects of subsequent steps in the signaling pathway frequently complicate the meaningful quantitative interpretation of the dose-dependent response. For example, while occupancy of a certain minimal number of receptors by an agonist may cause a proportional response, a later step in the pathway may become limiting at some greater level of stimulation. Further receptor occupancy then produces no additional effect. Thus, a plot of the drug's effect versus log concentration will lie to the left of a plot of fractional binding; drug potency will be greater than predicted by receptor affinity (Figure 2–5, *D*). This situation, in which a maximal apparent effect is achieved when a relatively small fraction of receptors is occupied, is explained by the concept of *spare receptors.* In at least some of these cases, a certain number of receptors can be lost (*e.g.*, with an irreversible antagonist) without diminution of the maximal observable response. Note that the existence of spare receptors does not necessarily imply a molecular excess of receptors over effector or transducer proteins. Spare receptors frequently are encountered whenever a receptor acts catalytically rather than stoichiometrically. In the case of the receptors that are tyrosine protein kinases, a few agonist-occupied receptors may be sufficiently active to maintain the phosphorylation of a greater number of substrate protein molecules. Similarly, a single G protein–coupled receptor can maintain the activation of hundreds of G protein molecules in some settings.

Because cellular signal transducing pathways are designed to amplify and integrate a multiplicity of stimulatory and inhibitory signals, it should come as no surprise that the outcome of pharmacological intervention generally is a complex consequence of the proximal effect of a single drug at its receptor. Increasingly complex mathematical models can be derived to describe the phenomenologic be-

havior of such systems, but analysis of the individual steps in the receptor–response pathway at a molecular level provides a more fruitful approach to identify new molecular targets for drug therapy.

PROSPECTUS

The continuing identification and expansion of molecular families for receptors in parallel with the accelerating discovery of their detailed mechanisms of action offers new therapeutic opportunities, just as refined insights into cell signaling pathways suggest new targets for specific disruption or enhancement of cellular function beyond receptor occupancy. These multiple potential drug targets coupled with the enormous potential for generating new molecules with combinatorial chemistry (Alper, 1994) or recombinant DNA strategies forecast a new era of diversity and specificity in therapeutic intervention.

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